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Research Article

In-vivo Hepatoprotective Evaluation of *Sicyos edulis* on Wistar Albino Rats

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ABSTRACT

The liver is known for synthesising enzymes, metabolism, and excretion of drugs and food. However, during biological processes, the abnormality occurs in the liver, which becomes a significant global health burden in humans, characterised by loss of synthetic function, breakdown of blood, irregular vitamin K, and localised, permanent changes to parenchymal cells. The study was designed to research the Phytochemical and biological screening of *Sicyos edulis* leaf for hepatoprotective activity on laboratory animals using paracetamol and methotrexate model for acute incidence. The study evaluated liver toxicity in healthy Wistar albino rats using two *in vivo* models. Each study group consists of six animals. In the first model, paracetamol p.o. for seven days. Similarly, in the second model, methotrexate was administered (single dose treatment) to animals with 20mg/kg, b.w., p.o. Both models were challenged with methanolic extract of *Sicyos edulis* leaf (MESEL) of doses 100mg/kg (low) and 200 mg/kg (high) p.o. for seven days, respectively. On day 8th, the blood samples were collected from the tail vein and analysed for various biochemical parameters. MESEL successfully restored the elevated serum biomarker levels in our study. The decrease in aspartate aminotransferase was observed by removing toxic metabolites, the reduction in alanine aminotransferase was due to an increase in ATP synthesis in mitochondria, thereby modulating the balance of liver energy metabolism, and the decrease in alkaline phosphates is due to tissue regeneration, an increase in total protein denotes the restoration of protein imbalance from acute liver injury. At different concentrations, all these effects strengthen the liver, regulate body metabolism, and ultimately inhibit further liver cell damage in favour of their regeneration. Our study also evidences the protective action of MESEL in rats against the Paracetamol and methotrexate model. The study reveals hepatocyte regeneration followed by hepatic restoration in pre-clinical settings.

Keywords: Acute liver disease; Sicyos edulis; Silymarin; Methotrexate; Liver enzymes

INTRODUCTION

Liver disease, a multi-etiological lethal complication, has steadily become one of the significant threats to public health. Liver disease is a substantial global health burden and a leading cause of morbidity and mortality worldwide. It develops with non significant clinical signs or symptoms. Hepato complications are observed in the immune system, body homeostasis, bile formation, protooncogene, and liver-related difficulty[1]. On the progression of liver disease, there is a significant elevation of recruited active hepatic macrophages that mark the role of innate immune cells[2]. Inflammatory liver illnesses are characterised by recruitments of neutrophil, eosinophil, glutamate, proinflammatory interleukins, nitric oxide, and macrophagic Kupffer cells (kcs), which later cause acute cell fibrosis and uncertain death[3]. Various toxins such as drug intoxication (e.g., acetaminophen overdose), viral or autoimmune hepatitis, Wilson's disease, and Budd-Chiari syndrome can all lead to acute liver failure (ALF), including loss of standard histological architecture, decreased cell mass, and decreased blood flow[4]. As a result, functional liver capacity gets damaged[5]. All negative impact on the liver was seen due to copious consumption of alcohol, abnormal accumulation of adipose tissue, and external insults followed by an imbalance of defensive factors such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gama glutamyl transferase (GGT) or alkaline phosphatase (ALP)[6]. Levels of serum AST or ALT are usually increased up to 1.5-

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to 4-fold but rarely exceed five times the upper limit of normal in the setting of non-alcoholic fatty liver disease (NAFLD). GGT and ALP levels may also be elevated. However, serum prothrombin time, bilirubin level, and serum albumin level are average, except in patients with NAFLD-associated cirrhosis[7]. According to World Health Organization 2018, alcohol consumption is responsible for three million deaths globally yearly, accounting for roughly 14% of overall mortality among persons aged 20 to 40[8]. Approximately 2 million people die yearly from liver disease, one million from cirrhosis complications, and one million from viral hepatitis and hepatocellular cancer (HCC)[9]. Non-alcoholic fatty liver disease (NAFLD) is a growing health concern, with an estimated global prevalence of 25%. According to a recent modelling approach, NAFLD cases in the United States would increase from 83 million in 2015, or about 25% of the population, to 100 million in 2030, or more than 33% of the population[10].

Treatment options for common liver diseases are limited and under clinical trials. Conventional or synthetic drug therapy used in treating liver diseases may lack efficacy and sometimes have serious adverse effects[11]. The effectiveness of treatments such as corticosteroids and interferons is inconsistent, carries the risk of adverse events such as ecchymosis, thinning of hair, acne, mild hirsutism, flu-like symptoms, haematological toxicity, and is often too costly[12]. Other anti-TB drugs such as Isoniazid, rifampicin, and pyrazinamide can also bring significant adverse effects such as neurological disorders, skin reactions, and gastrointestinal and hepatotoxicity[13]. It was reported that interferons could cause adverse effects such as headache, skin eruption, and influenza-like symptoms during treatment with some hepatoprotective drugs such as Bicyclol. Lamivudine can induce mitochondrial toxicity[14].

Therefore, in the absence of a reliable liver protective drug in modern medicine and the view of severe undesirable side effects of synthetic drugs, there is a growing focus on following systematic research methodology to evaluate the scientific basis for traditional herbal medicines for the treatment of liver disorders that are claimed to possess hepatoprotective activity. As a result, they are gaining importance in India and worldwide for their long-lasting curative effect, easy availability, natural healing, and fewer side effects[15].

One of the plants, *Sicyos edulis*, is believed to cure liver disease. The world's "Plant list" data also supports this proposed investigation due to their ethnopharmacological evidence and traditional therapeutical utilisation. This plant is also used as a vegetable and fruit in most habitats in north-eastern India and the mainland[16]. It is a herbaceous perennial climbing plant with tendrils and tuberous roots, cultivated since pre-Colombian times in Mexico. It is reported that the plant *Sicyos edulis* belongs to the Cucurbitaceae family and is used for treating dysentery, various uterus problems and inflammations, burning sensation, fatigue, various liver disorders, Jaundice, etc. So, *Sicyos edulis* was selected and aimed to establish the action and potential against the laboratory parameters such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total protein for rapid onset hepatic disease[17].

MATERIALS AND METHODS

Chemical and reagents

Petroleum ether, Methanol, Dragendorf's reagent, Fehling's solution A, Fehling's solution B, Benedict's reagent, and Chloroform were analytical grade and purchased from SD Fine Chem Limited. Paracetamol, silymarin and Methotrexate were purchased from Yarrow Chem Product, and the kits for the assay of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein were provided from Sigma Aldrich.

Experimental animals

The study was carried out on Wistar albino rats (160-200 g) of either sex. They were allowed to take standard pellet food and water. Before the experiment, the rats were kept in normal environmental conditions with room temperature 25-27°C relative humidity (55 ± 5) % and 12 h light/12 h dark cycle. IAEC permission was obtained before Animal experimentation with reference no HPI/2023/60//IAEC/PP-0199.

Selection, identification, authentication and collection

The entire plant *Sicyos edulis* was collected in September 2022 from Duga, East Sikkim. The leaves were washed thoroughly with tap water to segregate them from the mud and other extraneous material. The plant was dried at room temperature, away from direct sunlight. A herbarium A, the leaves of *Sicyos edulis*, was prepared and authenticated by the Botanical Survey of India, Gangtok, and Sikkim, and specimen no. 21HMPL054 was provided.

Preparation of extract

The dried leaves were milled to a coarse powder, and the extraction process was carried out using the Soxhlet apparatus using methanol as a solvent at 55-60°C. Then, the obtained extract was subjected to vacuum evaporation using a rotary evaporator to facilitate solvent recovery. Then, the section was received in a closed container to avoid moisture contamination[18].

Phytochemical analysis

The preliminary phytochemical analysis was done in the Methanolic extract of *Sicyos edulis* leaves (MESEL), which

was dissolved in water to determine the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, and tannins[19].

Antioxidant assay

DPPH free radical- scavenging activity assay

The *Sicyos edulis* extract's antioxidant activity was measured by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. Different volumes of plant extract were made up to 40μl with DMSO, and 2.96ml DPPH solution was added. The reaction mixture was incubated in the dark at room temperature for 20 minutes. Then, the absorbance was measured at 517nm against a blank. The free radical scavenging activity of the plant extract was determined by comparison with ethanol control[20].

In vivo study

Acute toxicity study

An acute toxicity study was conducted on *Sicyos edulis* leaf extract on Wister albino rats. The analysis was performed, followed by OECD TG-423. As per the guidelines, animals were treated with an initial dose of 5mg/kg, 50mg/kg, 1500mg/kg, and 2000 mg/kg. Without signs of mortality and severe morbidity, they would be subjected to a limit test for 2000-5000mg/kg. In the end, either NOAEL will be established, or, If causality, LD_{50} will be determined[21].

Paracetamol induced hepatotoxicity

The study duration of the Paracetamol-induced rat model was seven days. Five groups were made to carry out this model, each comprising six healthy Wistar albino rats. Normal control or negative control group of animals were treated with normal saline 0.9% v/v till 7 days; toxic control or positive control groups were treated with 1gm/kg body weight paracetamol., per oral, single-dose/day for seven days. Paracetamol was administered in the morning hour at 10 a.m. The standard group of Paracetamol-induced animals was challenged with 50mg/kg b.w. Silymarin per oral, single dose/day for seven days. At first, Paracetamol was administered. Then, in the evening, at 4 p.m., silymarin was given. Similarly, in the morning hour, Paracetamol was induced. First, a *Sicyos edulis* leaf extract of 100, 200 mg/kg of duos were treated with low and high doses, per oral, single-dose/day for seven days, respectively[22].

After 24hrs of the end treatment, i.e., on the 8th day, the blood samples were collected from the tail vein. After blood collection, the blood was allowed to clot for one hour at room temperature, and serum was separated by centrifugation at 2500 rpm at 30°C for 15 min. The serum was then collected and analysed for various biochemical parameters such as AST, ALT, ALP and T.P[22].

Methotrexate-induced liver toxicity

The experimental duration of the Methotrexate-induced rat model was seven treatments. In this acute model, Methotrexate was used on the first day to cause hepatic toxicity in each study group, excluding Negative control. Five groups were made to carry out this model, each comprising six healthy Wister albino rats. Normal control or negative control group of animals were treated with normal saline 0.9% v/v till 7 days; toxic control or positive control groups were treated with Methotrexate 20mg/kg b.w., per oral, single dose on starting day, observed behavioural, physiological, biochemical changes for seven days. In addition, the standard group of Methotrexate induced single day treatment animals was challenged with 50mg/kg b.w. Silymarin per oral, single dose/day for seven days. In addition, *Sicyos edulis* leaf extract of duos doses 100, 200 mg/kg treated with Low dose group and high dose group of Methotrexate-induced animals, per oral, single-day treatment[23].

RESULT

In vivo study

Acute toxicity study

The acute toxicity study reported no signs and evidence in laboratory animals. Therefore, per OECD TG-423, from NOAEL of 2000 mg/kg, low and high doses were calculated as 100 and 200 mg/kg, respectively, followed by safety factors 1/10 and 1/20.

Phytochemical analysis

The preliminary phytochemical analysis on the MESEL showed the presence of carbohydrates, reducing sugar, flavonoids and tannins. Table 1 represents plant metabolites.

Table 1: Results of Qualitative estimation

Phytoconstituent	Indication
Alkaloids	-
Carbohydrates	+
Reducing sugars	+
Anthraquinones	-
Flavonoids	+
Glycosides	-
Saponins	-
Steroids	-
Tannins	+
Protein	-

(+) indicates the presence of phytoconstituents, (-) indicates the absence of phytoconstituents

Antioxidant Assay

The antioxidant assay took different volumes of *Sicyos edulis* leaf methanolic extract. They were found to possess higher radical scavenging, reducing power and antioxidant activities and can inhibit lipid peroxidation. The % free radical scavenging activity of the MESEL was found to be 76%.

Effect of Sicyos edulis leaf on Paracetamol (PCM) induced liver toxicity

Paracetamol-induced hepatic injury was carried out for seven days. On the 8th day of treatment, serum was collected through the tail vein in a heparin tube; serum was analysed for all study animals for AST, ALT, ALP, and T.P. In the AST study, the standard control group of animals shows (34.44±1.10). There were significant changes reported in toxic control groups in terms of physiological and biochemicals, same found as (82.02±2.69) as compared ($p<0.001$) to the Normal control group. Silymarin at 50mg/kg reduced elevated AST to (40.09±0.93) compared to the toxic control group, but a relatively positive result was achieved compared to the normal group. A low dose of MESEL resulted (63.19±1.76), which was highly significant in comparison ($p<0.001$) to both the Normal control and toxic control group. However, Low doses were found to be less effective than silymarin. At a high MESEL yield (46.98±1.31), which is more effective than Toxic, and Low dose, but close adequate to silymarin.In the current study of ALT, the normal control group of animals demonstrated (36.51±0.59) on the 8th day. The toxic control group treated with Paracetamol for seven days shows a highly elevated ALT serum level (67.99±3.58) compared to standard, low and high doses of experimental animal groups due to hepatic damage. Whereas, silymarin at 50mg/kg b.w. shows theoretically resembles the normal control group due to its hepatoprotective activity (40.64±0.68). A low dose of MESEL shows (53.22±2.07) highly significant ($p<0.001$) values when compared to normal and toxic control groups. Much MESEL shows fundamental values (46.78±1.42) compared to the poisonous control and low-dose groups. After 24hrs, at the end of the treatment period, i.e., on the 8th day, evaluation of ALP was determined where the Normal control group of animals showed (82.46±0.53). In contrast, the toxic control group substantially increases ALP levels (205±9.35). The standard group treated with Silymarin at a dose of 50mg/kg (89.14±1.60) was non significant to the normal group of animals and highly significant to the toxic control group. In both low and high doses of MESEL, the animals showed a drop-down of ALP levels in a dependent manner. In this current study, the increased amount has shown potential positive results compared to the toxic and low-dose groups, and in the evaluation of T.P., the toxic control group treated with Paracetamol resulted in a decreased level of T.P. due to hepatic damage (2.28±0.18) caused by inducing Paracetamol when compared to the normal control group (7.88±0.23). The standard drug, Silymarin, shows reasonable levels (6.84±0.41) compared to the normal control group that received saline 0.9% v/v. However, animals that received MESEL at low (4.01±0.19) and high doses (6.03±0.44$^{\#}$) increased T.P. levels compared to the toxic control group that received Paracetamol at dose 1g/kg b.w. Table 2 depicts the effect of MESEL on AST, ALT, ALP, and T.P. Table 2 represents the MESEL against paracetamol-induced liver toxicity.

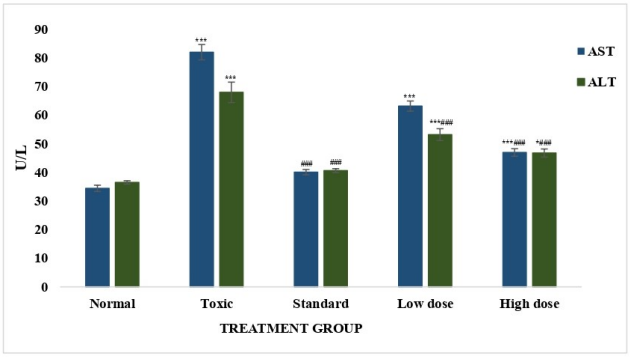

Fig. 1: Effect of *Sicyos edulis* leaf on Aspartate aminotransferase and Alanine aminotransferase

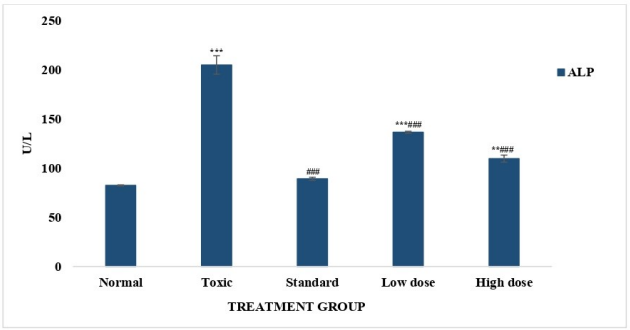

Fig. 2: Effect of *Sicyos edulis* leaf on Alkaline phosphatase

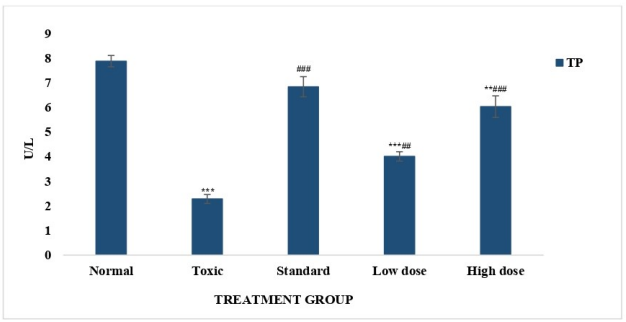

Fig. 3: Effect of *Sicyos edulis* leaf on total protein

Table 2: Effect of *Sicyos edulis* leaf against Paracetamol induced liver toxicity

Treatment	AST	ALT	ALP	TP
Normal control	34.44 ± 1.10	36.51 ± 0.59	82.46 ± 0.53	7.88 ± 0.23
Toxic control	82.02 ± 2.69***	67.99 ± 3.58***	205 ± 9.35***	2.28 ± 0.18***
Standard	40.09 ± 0.93$^{\#\#\#}$	40.64 ± 0.68$^{\#\#\#}$	89.14 ± 1.60$^{\#\#\#}$	6.84 ± 0.41$^{\#\#\#}$
Low dose	63.19 ± 1.76$^{***\#\#\#}$	53.22 ± 2.07$^{***\#\#\#}$	136.5 ± 0.99$^{***\#\#\#}$	4.01 ± 0.19$^{***\#\#}$
High dose	46.98 ± 1.31$^{***\#\#\#}$	46.78 ± 1.42$^{*\#\#\#}$	109.7 ± 3.56$^{*\#\#\#}$	6.03 ± 0.44$^{**\#\#\#}$

All the results were expressed as Mean ± SEM (n=6). *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Normal control; $^{\#}$P< 0.05, $^{\#\#}$P< 0.01, $^{\#\#\#}$P< 0.001 as compared Toxic control.

Effect of Sicyos edulis leaf against Methotrexate induced liver toxicity

Methotrexate caused hepatic injury by depletion of purine and pyrimidine synthesis in the liver cells. The model was carried out for seven days. On the 8th day, serum was collected through the tail vein in a heparin tube; all study animals were analysed for AST, ALT, ALP, and T.P.

In evaluating AST, the average control group yields (91.07±1.97). The toxic control group, where Methotrexate was used to induce hepatic toxicity, which is highly significant, shows an increase in AST levels (190±3.48) as compared to (p<0.001) normal control, which received saline 0.9% v/v every day till 7days. Silymarin at 50mg/kg achieved hepatoprotective activity by decreasing the AST level (97.05±2.11) compared to the toxic control and showed identical results to the normal control group. A low dose of MESEL resulted (125.85±4.86), which offers relative values (p<0.001) when compared to normal control and toxic control. But found to be less effective when compared to Silymarin. At a high dose, MESEL shows (113.69±5.55) better results when compared to poisonous control, a low amount but yields similar AST levels to silymarin.

In this current study, the ALT serum biomarker of standard control shows (80.41±1.46). There were significant changes in toxic control (140.5±11.5) in terms of physiological and biochemicals when compared to the (p<0.001) normal control group. Silymarin at a dose of 50mg/kg shows its hepatoprotective property by significantly decreasing (85.35±1.25) ALT levels compared to the toxic control group. However, it yields identical values to the normal control group. A low dose of MESEL shows slightly similar results (120±10.3) to poisonous control. The animals were treated with Methotrexate on the first day to induce hepatic toxicity compared to the normal groups. At the same time, a high dose of MESEL yields (105.4±1.35) shows more effectiveness than the toxic group but demonstrates slightly significant values compared to the normal control group.

In evaluating ALP, the normal control group animal's results (105.4±1.35). The toxic control group showed hepatotoxicity yields (153.2±9.17), which is highly significant as compared to the (p<0.001) normal control group. Standard drug Silymarin at a dose of 50mg/kg per oral reduced ALP levels (87.11±2.01) due to its existing hepatoprotective action. A low MESEL resulted in (138.54±8.08), which was highly significant compared to standard control and toxic control. At a high dose of MESEL, the levels of ALP were slightly similar to the regular control group but showed high significance when compared to the toxic control group.

Similarly, in this current result, the levels of T.P. in the regular control group show (9.0±0.16). At the same time, the animals treated with Methotrexate per oral yield decreased in T.P. (2.9±0.22) compared to the normal control group that received saline 0.9% v/v for seven days. However, at 50mg/kg, Silymarin showed hepato-protective activity by increasing T.P. levels (8.06±0.19).

A low dose of MESEL shows a highly significant increase (153.2±9.17) in levels of ALP as compared to standard control and toxic control. Still, more MESEL results are slightly necessary (112.4±5.21) in ALP levels compared to what was expected. In addition, it yields highly significant values compared to the toxic control group, demonstrating closed values with the standard group. Table 3 depicts the MESEL against methotrexate-induced liver toxicity.

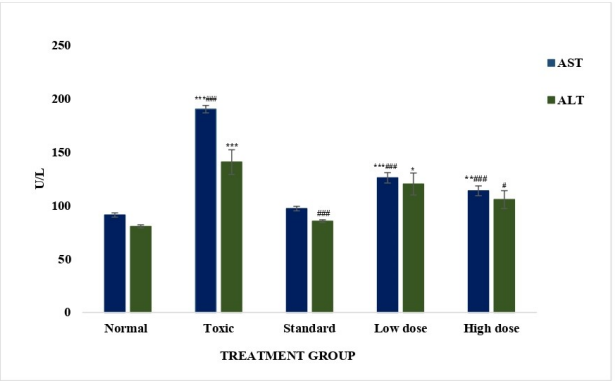

Fig. 4: Effect of *Sicyos edulis* leaf on Aspartate aminotransferase and Alanine aminotransferase

DISCUSSION

The present study aimed to carry out the hepatoprotective activity for *Sicyos edulis* leaf on Wister albino rats, followed by a paracetamol-induced model and a Methotrexate model. The collection of *Sicyos edulis* leaves was executed during

Table 3: Effect of *Sicyos edulis* leaf against Methotrexate-induced liver toxicity

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	T.P. (U/L)
Normal control	91.07 ± 1.97	80.41 ± 1.46	105.4 ± 1.35	9.0 ± 0.16
Toxic control	190 ± 3.48***	140.5 ± 11.5***	153.2 ± 9.17***	2.9 ± 0.22***
Standard	97.05 ± 2.11$^{\#\#\#}$	85.35 ± 1.25$^{\#\#\#}$	87.11 ± 2.01$^{\#\#\#}$	8.06 ± 0.19$^{\#\#\#}$
Low dose	125.85 ± 4.86$^{***\#\#\#}$	120 ± 10.3*	138.54 ± 8.08$^{***\#\#\#}$	5.69 ± 0.23***
High dose	113.69 ± 5.55$^{**\#\#\#}$	105.4 ± 1.35$^{\#}$	112.4 ± 5.21$^{*\#\#\#}$	6.63 ± 0.32$^{*\#\#}$

All the results were expressed as Mean ± SEM (n=6). *P< 0.05, **P< 0.01, ***P< 0.001 as compared to Normal control; $^{\#}$P< 0.05, $^{\#\#}$P< 0.01, $^{\#\#\#}$P< 0.001 as compared Toxic control.

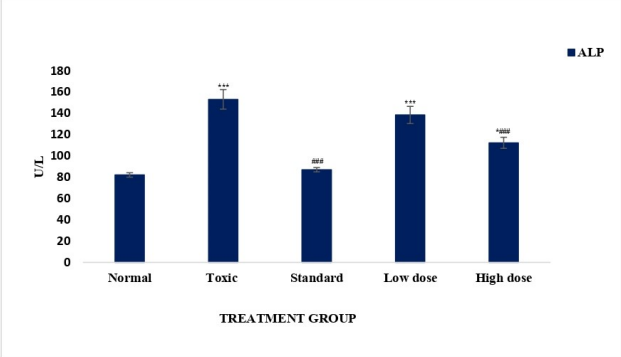

Fig. 5: Effect of *Sicyos edulis* leaf on Alkaline phosphatase

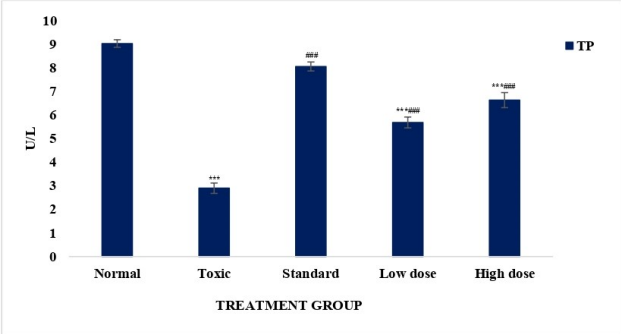

Fig. 6: Effect of *Sicyos edulis* leaf on total protein

September from the Duga region, East Sikkim, in September. The study was initiated with the authentication of *Sicyos edulis*, belonging to the family Cucurbitaceae. The leaves previously kept for drying were coarsely powdered and subjected to Soxhlet extraction to facilitate the extraction process. The solvent that was used for the extraction was Methanol. Based on the literature review, methanol was an ideal solvent for extracting the flavonoid and polyphenol compound, the main phytoconstituent of interest for the expected pharmacological activity in future work[24]. The 200gm of coarsely powdered plant material gave a yield of 17.4%. The previous literature review confirmed alkaloids, phenols, flavonoids, saponins, proteins, carbohydrates, and tannins in the *Sicyos edulis* leaf extract[25]. The extract's phytochemical estimation revealed the presence of flavonoids, carbohydrates, reducing sugars, and tannins in the section. However, the presence of alkaloids, saponins, and proteins was not determined through the phytochemical estimation, which can be due to solvent selection or the variety of the fruit. The source and location from which the plant was collected can also impact the phytoconstituent in the particular plant. The number of phytochemicals in the extract gives the various pharmacological activities an advantage. Plants can be used to treat various diseases as they contain many phytochemical constituents, such as phenolic and flavonoid compounds, which exhibit a variety of pharmacological activities. These plants can be used in several ways to promote and maintain good health in an individual. Present research includes the presence of carbohydrates, in which restriction plays a significant role in the modulation of lipid metabolism by stimulating lipase enzymes[26]. Furthermore, carbohydrates improve NAFLD compared with low-fat diets related to liver diseases. So, the presence of carbohydrates might influence MESEL on liver protection[26]. Reducing sugar in the current investigation indicates the pharmacodynamic property of *Sicyos edulis*; here, pharmacodynamic properties refer to MESEL, affinity towards receptors, protein of interest, and genetical expression[27]. The extract has phenolic and flavonoid compound, which is known to exhibit antioxidant property and helps reduce the various reactive oxygen species formation. ROS is said to be involved in the pathogenesis of different diseases, and the case of liver disease, ROS plays a vital role in its development[28]. The current investigation on *Sicyos edulis* reports the presence of tannins. The part of tannin was also established in earlier hepatoprotective studies. This includes significantly reducing serologic enzymes such as ALT, AST, ALP, and T.P.[29]. Therefore, it is speculated that the extract of *Sicyos edulis* leaf can help alleviate the development of liver disease by inhibiting the production of ROS and other factors involved in the progression of the disease. The antioxidant assay was performed by using DPPH. The % radical scavenging activity of the plant extract was 3.6%.

Therefore, in the proposed research, the hepatoprotective activity of the methanolic extract of *Sicyos edulis* leaf will be evaluated on Paracetamol and induced liver disease. This proposed investigation will build a bridge between chayote's

pharmacodynamics and pharmacokinetics in pre-clinical studies on experimental laboratory animals.

The preliminary study of hepatoprotective activity was carried out for *Sicyos edulis* leaf, followed by an *in vivo* paracetamol model. Paracetamol was administered with a dose of 1gm/kg body weight subjected to develop hepatic damage in Wister albino rats.

After administering Paracetamol orally, its absorption was delayed due to food entrapment in the stomach and extended the peak plasma concentration with overdose. The metabolism occurs in the liver in phases: Phase I, II and III. Usually, 90% of the drug is metabolised in the Phase II reaction and excreted through urine[30]. Still, the remaining 10% of the drug metabolised in Phase I response and produces a highly reactive metabolite called as N-Acetyl P-benzoquinone imine molecule, which impacts glutathione and increases free radicals such as hydrogen oxide, hydrogen superoxide, and nitric oxide and suppresses the oxidation reaction at mitochondria to suppress the ATP synthesis that will further cause damage to DNA of the mitochondrial cell, protein unfolds, and cell necrosis. It also releases mitochondrial contents such as apoptosis, karyolysis, vacuolisation, and inflammation[31]. These inflammatory cascades elevate injury to the liver and its enzymatic functions at the cellular and molecular levels. In our present investigation, Paracetamol causes severe liver cell damage, as reported on the 8th day of laboratory parameters. There was an elevated level of AST in all the animals except the negative control highlighted in Table 1. An increase in AST level is often a sign of liver disease. An increased AST level is observed in serum due to scarring of the liver, permanent damage of liver tissue, myocardial infarction, hemochromatosis, liver ischemia, uncontrolled growth of liver cancer, and consumption of toxic drugs to the liver, primarily physically dependent drugs[32]. An ALT blood test aims to help evaluate the liver's health. If cells in the liver are damaged, it can cause ALT to leak into the blood, so an ALT blood test can help find liver issues; risk factors associated with rising ALT could be the intake of more alcohol, Family history, diabetes, Obesity, improvement in ALT levels have shown that the standard Silymarin drug exhibited its hepatoprotective activity[33]. ALP is an enzyme that's found throughout the body. ALP blood tests measure the level of ALP in the blood that comes from the liver and bones, and it's one of the tests included in a comprehensive metabolic panel. High levels of ALP in the blood may indicate liver disease or certain bone disorders. ALP is often considered a liver enzyme because its primarily found in the liver. However, it also exists in the following places: the Bile duct, Bones, kidneys, Intestines, and Placenta in pregnant people; abnormal levels of ALP in the blood can reflect damage to tissues or disruption of normal bodily processes[34]. The TP test measures the total amount of two proteins found in the fluid portion of blood. These are albumin and globulin. Proteins are essential components of all cells and tissues. Albumin helps to keep fluid from leaking out of our blood vessels. It also transports chemicals in the blood. Globulins are an essential part of the immune system. Low total protein may indicate bleeding, liver disorder, malnutrition, and inflammatory conditions[35].

In our current investigation, statistics reveal that silymarin can restore elevated AST and ALT and control the protein breakdown highlighted in Table 2. The recovery of biochemical parameters supported by Pharmacokinetic studies with silybin-phosphatidylcholine complex has shown an increase in the oral bioavailability of silybin in healthy human subjects, probably by a facilitatory role of drug complex on the passage of the drug across the gastrointestinal tract. Silymarin showed histopathological evidence of hepatoprotection by preventing hepatic cell necrosis or regeneration[36]. Silybin di- hemi succinate, a soluble form of silymarin flavonoid, has protected rats against liver glutathione depletion and lipid peroxidation induced by acute acetaminophen hepatotoxicity potential benefits of silymarin as an antidote. Scientists discovered that silymarin treatment normalised the elevated biochemical parameters of the liver and serum caused by acetaminophen by stabilising the plasma membrane *in vitro* studies on rat hepatocytes[37].

The studied plant, *Sicyos edulis*, comprises rich secondary metabolites such as carbohydrates, flavonoids, reducing sugar, and tannins, which are also shown in Table 1; this is attributed to the hepatoprotective property of plant in paracetamol-induced liver injury at both doses 100, 200mg/kg as low, and high doses respectively for reducing elevated ALT, AST, ALP, and inhibiting proinflammatory protein breakdown. Furthermore, several pharmacological studies concluded that the *Sicyos edulis* plant helps the body from free radical damage and slows down the progression of hepatic tissue impairment caused by inducing the Paracetamol model. A previous study reported the ethanolic extract of *Sicyos edulis* roots against Paracetamol-induced liver damage in rats at 300 and 600mg/kg. It was revealed that the elevation of liver serum biomarkers AST, ALP, and ALT could reverse the hepatic damage to normal and increase the T.P., which supports the hepatoprotective activity of the *Sicyos edulis* plant[17]. However, we have challenged the plant *Sicyos edulis* leaf with methanolic extract and different fractions of *Sicyos edulis* (100 and 200mg/kg). It was found to reduce the levels of liver serum biomarkers effectively. Furthermore, our study recorded the higher relative potency at a high dose of *Sicyos edulis* compared to silymarin.

Methotrexate is well-known to cause serum aminotransferase elevations and long-term therapy has been linked to the development of fatty liver disease, fibrosis and even cirrhosis. In addition, with high-dose intravenous Methotrexate, serum markers will be imbalanced, resulting in indications of liver damage by depletion of purine and

pyrimidine synthesis in the liver cells[38].

The current acute model also supports the Methotrexate to induce hepatic toxicity even at a single dose, which was progressively increased till the 7th day with clear signs of black, tarry stools, itching, rash, reddening of the skin, swelling of the eyelids, hands, feet or lower legs.

With the repeated dose of Silymarin, chayote methanolic extract, significant recovery was observed in clinical signs, symptoms, and evidence yields from laboratory parameters restoration such as AST, ALT, ALP, and total protein. In addition, Silymarin shows reproducible results against methotrexate-induced liver injury at 50mg/kg compared to complicated hepatic animals, and recovery under silymarin was well visible.

This made *Sicyos edulis* a permissible candidate for further hepatotoxicity studies, including special examinations such as carcinogenicity, mutagenicity, and reproductive, and Tier I studies for long-existing studies.

CONCLUSION

The Cucurbitaceae family of chayote is widely accepted as a vegetable in the Himalayan region and observed nationwide. Ethnobotany includes cardioprotective, anti-epileptic, anti-obesity, anti-microbial, and anti-diabetic. Chayote is a herbaceous climbing tree whose different parts, such as fruits, leaves, roots and stem, have been researched extensively for its medicinal benefits based on definitive evidence. The current study gave several phytochemicals such as carbohydrates, reducing sugar, polyphenols, flavonoids, and tannins. In the present investigation, our findings provided evidence to support *Sicyos eduli*'s protective effect against Paracetamol and methotrexate-induced hepatotoxicity. In addition, the results showed that the MESEL protected hepatocyte cells against injury by Paracetamol and Methotrexate. However, MESEL at a dose of 200mg/kg has shown a more significant effect on the development of Paracetamol and methotrexate-induced liver injury, which can facilitate the elevated levels of serum biomarkers AST, ALT, ALP and T.P. Our study also added therapeutic and nutritional value in Indian kitchens for food technology.

Thus, the current study confirms the methanolic extract of *Sicyos edulis* leaf protective action in rats against the Paracetamol and methotrexate model. Furthermore, the quote was very promising, as evidenced by the reversal of the altered values after administration, most likely by promoting hepatocyte regeneration, which restores integrity.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship Contributions

Concept: K.B., D.C., Design: K.B., D.C., Data Collection or Processing: K.B., D.C., Analysis or Interpretation: D.C., M.C., Literature Search: K.B., D.C., Writing: A.C., D.C., K.B., NR.B.

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